STAIN TECHNOLOGY

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A RAPID STAINING METHOD FOR THE MICROSCOPICAL EXAMINATION OF MILK.—A PRELIMINARY NOTE

DONALD B. SHUTT, *Bacteriology Department, Ontario Agricultural College, Guelph, Canada*

In the routine examination of milk with the Breed microscopical method, speed in the staining of the smear, coupled with a clear background are important factors if many samples are to be examined.

The standard method requires about seven minutes, more or less. The bacteria and other cells are stained dark blue against a light blue background. However, when sour milk is used, the heat required in the drying of the smear tends to curdle the milk on the slide, with the result that thick dark streaks are often present in the background. These thickened areas often conceal bacteria and other cells which may be present.

A method has been developed whereby the milk smear can be defatted, fixed and stained in one operation in from five to fifteen seconds and in which the background can be completely decolorized.

The method is based upon the rapid staining formula developed by Erb[1] and the principle of background decolorization of Mallman and Churchill.[2] The formula is as follows:

Ether	100 ml.
Methyl alcohol, absolute	100 ml.
Methylene blue	1 g.
Hydrochloric acid	0.7 ml.

The preparation of the formula is important. The ether and the methyl alcohol should first be mixed together before the methylene blue is added. As soon as the dye is in solution the hydrochloric acid can be included. Variations in this order of procedure may result in failure.

[1]Erb, N. M. A rapid stain for the direct microscopic examination of milk. J. Lab. and Clin. Med., **14**, 377–9. 1929.

[2]Mallman, W. L., and Churchill, E. A rapid test for determining bacteria in liquid eggs. U. S. Egg and Poultry Magazine, **48**, 406. 1942.

Best results are obtained when the solution is held in a stoppered bottle and in the refrigerator when not in use. The solution should be filtered before using.

The ingredients employed by the writer in the above formula were as follows: ether, C. P., Bakers Analytical; methyl hydrate, Analar, (i. e., methyl alcohol) British Drug Houses Ltd.; methylene blue, 187717/38823, British Drug Houses Ltd; hydrochloric acid, Analar, British Drug Houses Ltd.

The milk slide is prepared in the usual manner by employing either a loop or a pipette. The smear is dried carefully and is then dipped into the staining solution for the required time, removed, washed, dried and examined. Staining for fifteen seconds or more will give a background which is completely decolorized. This may present a problem in focusing the microscope, especially when good quality milk having few bacteria is employed. Experience has shown that an exposure of from five to ten seconds will leave a faintly colored background on which it is easy to focus the microscope. The bacteria and other cells are stained blue.

It is realized that this method should be compared with other rapid staining procedures for the same purpose, such as those of Newmann and of North. As time is lacking at present for this comparison, the present preliminary note is published for the benefit of anyone who wishes to make use of the proposed method.

A NEW CYTOLOGICAL TECHNIC, TANNIN-IRON III, FOR NUCLEOLI AND PLASTIDS

M. C. DE REZENDE-PINTO, *Bolseiro do Instituto para a Alta Cultura, Universidade do Porto, Portugal*

Tannin-iron III, as a technic, differs very little from Salazar's tannin-iron I, and must be considered as a new modification of the latter[1]. But the results obtained are very different and its use in plant cytology will be found very advantageous due to its specificity for certain cellular organs.

This specificity arises from the previous hydrolysis in normal hydrochloric acid. The time of hydrolysis depends on the composition of the fixing fluids. In this way, the nucleoli and the "grana" of the plastids acquire a specific secondary tannophyly although they are not at first tannophylic.

The procedure is as follows:

I—*Fixation*. Any of the following fixing procedures may be used: Carnoy (2 vol. absolute ethyl alcohol, 1 vol. glacial acetic acid); salted formalin (10% formalin in 3% aqueous sodium chloride); "acetoforsaldicr." (immersion for about 24 hours in 5% acetic acid; then for about 48 hours in salted formalin; rinsing for 24 hours in running water; and finally immersion for 3–6 days in 3% aqueous potassium dichromate).

II—Embedding in paraffin

III—Hydrolysis

1. Remove paraffin from sections and bring them down to distilled water.
2. Rinse in cold normal hydrochloric acid.
3. Hydrolyze in hot normal hydrochloric acid (58°–60° C.) for 4 minutes for material fixed in Carnoy's fluid and 2 minutes for material fixed in "acetoforsaldicr." or salted formalin.
4. Rinse in distilled water.

IV—Staining

1. Transfer sections to Salazar's acetic tannin in which they must remain for 5 minutes.
   (To prepare Salazar's acetic tannin, dissolve 6 g. tannic acid in 10 ml. glacial acetic acid and then add 30 ml. distilled water).

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[1]See: Salazar, A. L. 1944. La technique de tannin-fer. Stain Techn., **19**, 131–5. Id. 1946. A new technic, tannin-iron II. Id., **21**, 149–51.

2. Wash in distilled water.
3. Stain sections in 4% iron alum solution for about 5 minutes.
4. Wash thoroughly in running water.
5. Dehydrate and mount in Canada balsam.

QUICK STAINING METHOD FOR FROZEN SECTIONS

W. V. Cole, *Department of Anatomy, Kirksville College of Osteopathy and Surgery, Kirksville, Missouri*

Since the advent and general acceptance of frozen sections in histological and pathological laboratories it has been necessary to devise methods for staining these sections. The usual method is fixing the tissue to a slide by the use of celloidin. This paper is an attempt to describe a permanent, quick method of staining frozen sections without distortion or mechanical tearing of the tissues.

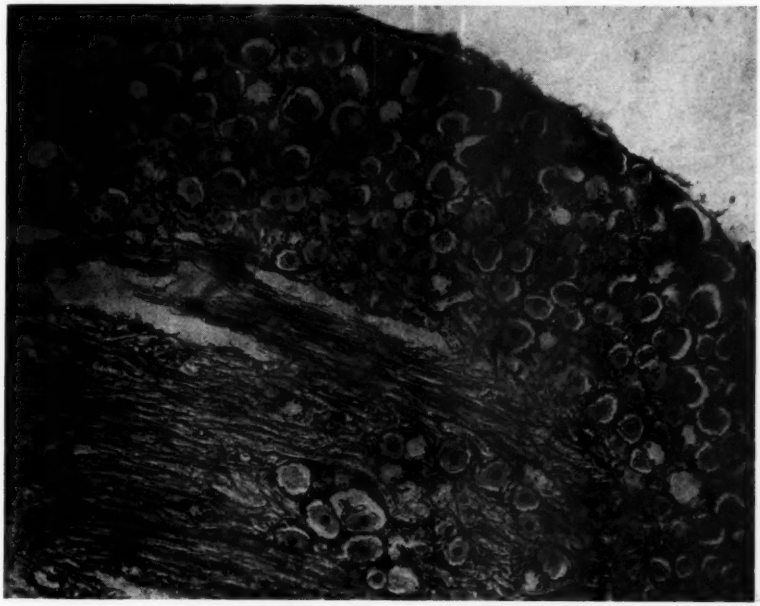


Fig. 1. Spinal Ganglion cells. Rat. ×450. Frozen section cut at 10 μ and stained with Mayer's alum hematoxylin and eosin.

The equipment necessary for this procedure consists of a glass 'spot plate' and a perforated section lifter of such a size as to fit one of the depressions in the spot plate.[1]

[1]Section lifter used by writer (purchased from A. S. Aloe Co., St. Louis) has a bowl with a top diameter of 18 mm. and a 163 mm. handle soldered to one side of the bowl at right angles.

Almost all of the common stains can be adapted to this method. Naturally, those methods which depend upon impregnation cannot be included, although after staining by them the tissues can be sectioned by the freezing method.

Procedure

The sections are cut from 5–15μ and placed in distilled water. The stains and reagents of the particular method to be used are placed in the spot plate in the order in which they are to be needed. As an example the hematoxylin and eosin method will be described here.

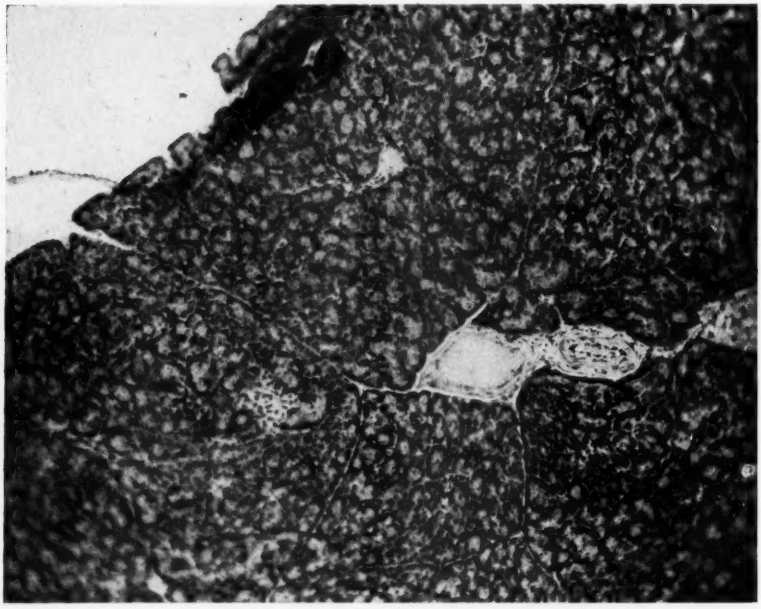


Fig. 2. Pancreas. Rat. $\times$160. Frozen section cut at 15μ and stained with Harris' hematoxylin and eosin.

In the first depression Mayer's alum hematoxylin[2] is placed. It requires but a few drops of solution to fill the depression. Following this the different concentrations of alcohol are placed in successive depressions. In this laboratory 25%, 35%, 50%, and 75% alcohol are used. In the following depression, 1% eosin in 75%

[2]Mayer's alum hematoxylin: Dissolve 1 g. hematoxylin in 1000 ml. distilled water. Add 0.2 g. sodium iodate and 50 g. ammonium or potassium alum. Add 1 g. citric acid and 50 g. chloral hydrate.

methyl alcohol is placed. This is followed by several changes of 95% alcohol and finally the clearing solution of creosote and beachwood oil is included.

A section of the tissue is picked up by the perforated section lifter and placed in the first solution in the spot plate. Because there is no embedding material in the tissue, staining is much more rapid than by other methods. In this laboratory it has been found that 15 seconds is long enough to stain the section, and 5 seconds is sufficient in each of the successive alcoholic solutions. Fifteen seconds in the clearing agent removes all excess stain and water.

In some cases the tissues are wrinkled after sectioning. This does not prevent staining and they will usually straighten out when placed in the cleaning agent. It is, however, sometimes necessary to straighten them by means of fine needles.

After clearing, the tissues are floated onto a clean glass slide and mounted in any of the common mounting media. Balsam is commonly used, but in this laboratory Clarite[3] has given better results.

Although the hematoxylin and eosin stain has been described it is possible to obtain consistent results with Mallory's triple dye stain, Masson's stain, and any of the single stains.

Sections made by this method of both fixed and unfixed tissues have given excellent permanent sections. Fig. 1 and Fig. 2 show tissues prepared by this technic.

[3]Synthetic resin sold by Central Scientific Company, Chicago, Illinois.

HISTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE IN GERMINATING MAIZE KERNELS

OTTO E. KUGLER, *Department of Zoology and Physiology*, AND EDMUND H. BENNETT, *General Biology, Division of General Studies, University of Illinois, Urbana, Ill.*

ABSTRACT.—**Sections of germinating maize kernels obtained by freezing technic were examined for acid phosphatase according to the histochemical method of Gomori. The embryonic axis, scutellum and aleurone layer were found positive for the enzyme. A microtechnical method for preformed phosphate, based on Sumner's colorimetric method for phosphorus, is described.**

INTRODUCTION

Save for the recent work by Glick and Fischer (1945a; 1945b; 1946) on phosphatases in wheat grains and sprouts, the application of the valuable technic of Gomori (1939; 1941) for the localization of phosphatases has been confined to studies of animal tissues. In the present investigation Gomori's method for acid phosphatase was adapted to a study of germinating maize kernels. The principle of Gomori's technic is as follows: Tissues are incubated with sodium glycerophosphate in the presence of lead nitrate. The phosphate liberated by the enzyme is trapped *in situ* by forming insoluble lead phosphate. The latter upon treatment with ammonium sulfide yields a brown to black deposit of lead sulfide, which affords a visual localization of sites of phosphatase activity.

METHOD

Fully-matured maize kernels were induced to germinate and softened for sectioning by soaking them in water at 38° C. for 24 hours. The kernels were then trimmed and sectioned at once, or allowed to develop for an additional period between moist blotters at room temperature before further treatment.

Trimming was done with a razor-blade by cutting a longitudinal slice from each side of a kernel so that the remaining central portion, from which sections through the median longitudinal plane are to be obtained, contained the scutellum and embryonic axis. In this operation the kernels must be handled carefully, for the slightest rolling or crushing will be evidenced later in imperfect sections.

The trimmed kernels were sectioned with a sliding microtome, equipped with a freezing attachment. For gross demonstration of

acid phosphatase, sections of 100 to 120μ in thickness were cut; while for cytological study sections of 60 to 80μ were used.

Two methods of handling the sections were tried. In the first method the sections were transferred with a wet camel-hair brush to square cover slips (two sections were placed on each cover slip). Three drops of 95% alcohol were added over each section, then drained off, and replaced with absolute alcohol. The latter, in turn, was replaced with 1% alcohol-ether collodion. By exposure to the air for a few minutes, the collodion hardened; and the cover slips with the affixed sections were placed in the incubating mixture.

While the above method gave good results and is recommended in dealing with either soft or friable material, it was found that the sections of corn kernels were rigid enough to permit them to be transferred directly with a section-lifter through the reagents, which were contained in 25 ml. Stender dishes. In view of this, the freshly-cut sections were fixed in absolute acetone for 15 minutes, rinsed in distilled water, and placed in the incubating mixture. Sections held overnight, however, in ice-cold acetone and then incubated, showed no discernible loss in enzyme strength.

Incubation was carried on in an oven at 38° C. for 45 minutes. Since pure α-sodium-glycerophosphate recommended by Gomori was not available, Moog's (1944) incubating mixture was used. It consists of the following ingredients mixed in the order given: acetate buffer at pH 4.7, 2½ parts; 5% lead nitrate, 1 part; distilled water, 15 parts; 2% sodium glycerophosphate (Eastman's 52% alpha and 48% beta), 3 parts. After standing overnight in a refrigerator the mixture was centrifuged to rid it of precipitated lead-β-glycerophosphate, and the clear supernatant was used as the incubating mixture.

The incubated sections were rinsed in three changes of distilled water for 2 minutes. To remove lead-protein and lead-glycerophosphate they were next rinsed in 2½% acetic acid for 4 minutes. This was followed by a rinse in three changes of distilled water for 3 minutes.

The sections were then placed for 2 minutes in a dilute solution of ammonium sulfide (1 ml. of Eimer and Amend's light ammonium sulfide to 50 ml. of distilled water).

After rinsing in several changes of distilled water, followed by tap water, the sections were dehydrated through 50%, 95%, and two changes of 100% alcohol. The sections were retained in each alcohol for 5 minutes, except for the second change of absolute alcohol in which they were held for 10 minutes.

Next the sections were placed for 5 minutes in a 1:1 mixture of

xylene and 100% alcohol. They were cleared by keeping them 5 minutes in a first and 15 minutes in a second change of xylene. Finally the sections were transferred to slides and mounted in Clarite.

If desired the sections may be counterstained during the dehydration process. Eosin Y (0.5 g. in 100 ml. of 95% alcohol) gave satisfactory results.

Sites of phosphatase activity stain black or brownish-black. Likewise, because of chemical interaction with lead nitrate, any preformed deposits of phosphate will stain similarly. The preformed phosphate will appear if glycerophosphate is omitted from the incubating mixture, while sites of phosphatase activity will not. Therefore control sections were treated like the experimental ones except that glycerophosphate was omitted from the incubating mixture. Thus control sections showed preformed phosphate while the experimental ones showed in addition phosphate liberated from the substrate by the enzyme. Slides prepared as described above showed no appreciable fading after three months.

The distribution of preformed phosphate in the control sections was found to be in agreement with that obtained by a microtechnical test for inorganic phosphate, suggested by Sumner's (1944) method for the colorimetric determination of phosphorus. With a razor-blade, thick free-hand sections were made of corn kernels. After 15 minutes fixation in absolute acetone, the slices were placed in a 1% ammonium molybdate solution for 15 minutes to allow the inorganic phosphate in the tissues to combine with the molybdate to form phosphomolybdic acid. Following a rinse in distilled water, the sections were examined with a dissecting microscope while immersed in a freshly prepared, acidified ferrous sulfate solution (5 g. $FeSO_4 \cdot 7\ H_2O$ and 5 ml. of 7.5 N H_2SO_4 made up to 50 ml. with distilled water). The blue-colored areas that appear within 5 minutes are caused by a reduction of phosphomolybdic acid to blue phosphomolybdous acid. Since the blue stain diffuses, the examination must not be delayed.

Results

Acid phosphatase occurs in all parts of the embryonic plant and in the aleurone layer (Fig. 1). It is not present in the pericarp, nor is it found in the endosperm except in regions contiguous to the epithelial layer and to the aleurone layer.

Embryonic axis—In the plumule, coleoptile, radicle, coleorhiza, and hypocotyl, the cytoplasm, as indicated by small brown-staining granules, has moderate amounts of acid phosphatase; the nuclei, however, are strongly positive. Control sections of the embryonic

EXPLANATION OF FIGURES

Al, aleurone layer
Ea, embryonic axis (radicle indicated)
En, endosperm
Ep, epithelial layer
P, pericarp
Sc, scutellum
SCS, storage cells of scutellum

Fig. 1. Illinois Hull-less, popcorn. Germinated 50 hours, 100μ. Stained for acid phosphatase. $\times 5$.

Fig. 2. Hybrid Golden Bantam, sweet-corn. Germinated 24 hours, 60μ. The storage cells of the scutellum and the epithelial layer are positive for acid phosphatase. $\times 290$.

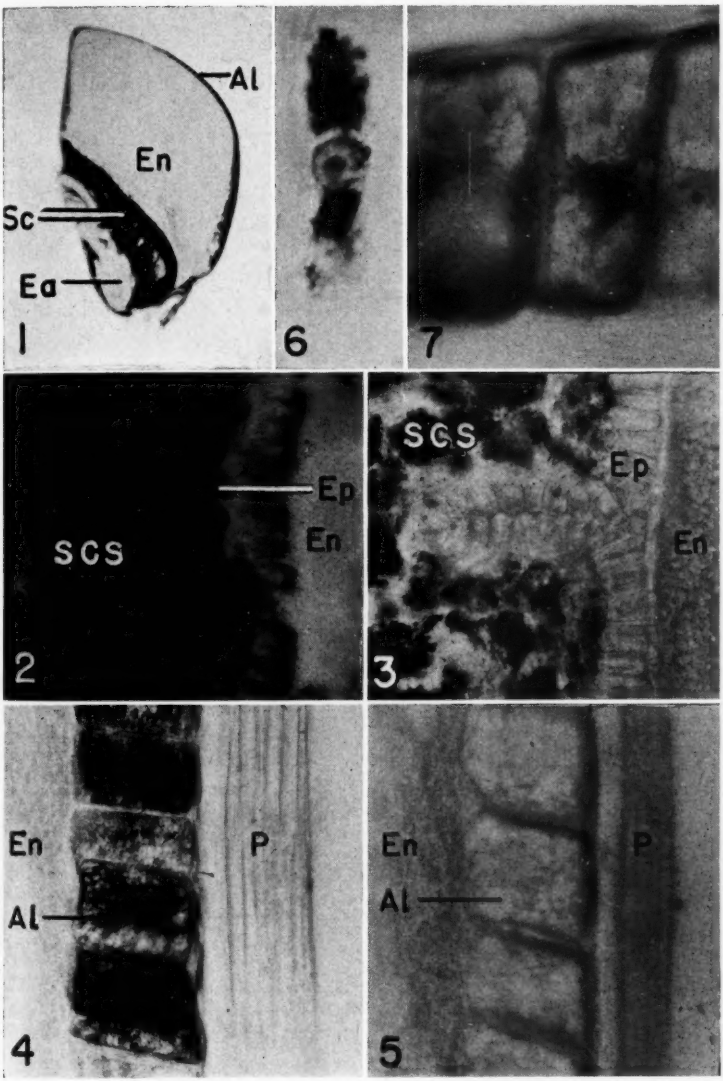
Fig. 3. Hybrid Golden Bantam, sweet-corn. Germinated 24 hours, 60μ. The storage cells of the scutellum are positive for preformed phosphate, but the epithelial layer is negative. $\times 290$.

Fig. 4. Hybrid Golden Bantam, sweet-corn. Germinated 50 hours, 80μ. The aleurone cells are positive for acid phosphatase. $\times 290$.

Fig. 5. Hybrid Golden Bantam, sweet-corn. Germinated 50 hours, 80μ. The aleurone cells are weakly positive for preformed phosphate. $\times 290$.

Fig. 6. Hybrid Golden Bantam, sweet-corn. Germinated 24 hours. The epithelial cell shows cytoplasm, nucleus, and nucleolus positive for acid phosphatase. $\times 1750$

Fig. 7. Hybrid Golden Bantam, sweet-corn. Germinated 50 hours. The aleurone cells show the cytoplasm, irregularly-shaped nuclei, and nucleoli positive for acid phosphatase. $\times 600$.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axis reveal a slight quantity of preformed phosphate, and it is found only in the cytoplasm.

Scutellum—In the storage cells of the scutellum, acid phosphatase is strongly marked by heavy black deposits in the cytoplasm. The epithelial layer of the scutellum is also positive and black cytoplasmic granules are most pronounced in the ends of the cells facing the endosperm (Fig. 2). In sections of 100μ or more in thickness a dark zone in the endosperm bordering the epithelial layer suggests a diffusion of the enzyme from the epithelial layer The nuclei of the storage cells and of the epithelial cells of the scutellum are positive; the nucleoplasm takes a diffuse brown stain, and a single nucleolus appearing in each nucleus stains black (Fig. 6).

Control sections show considerable deposits of preformed phosphate in the cytoplasm of the storage cells of the scutellum. The epithelial layer, however, is negative for preformed phosphate (Fig. 3). The aforementioned figure shows one of the characteristic invaginations of the epithelial layer into the storage tissue of the scutellum.

The abundance of acid phosphatase in the storage cells of the scutellum may be related to the abundant lecithin stored there. Glycerophosphate, resulting from the hydrolysis of lecithin, would serve as the normal substrate for the enzyme. The liberated phosphate, upon translocation to the meristematic tissue of the embryonic plant, would enter into the formation of nucleic acid and nucleoproteins.

Aleurone layer—The heavy-walled cells of the aleurone layer are strongly positive for acid phosphatase (Fig. 4). Black granules are present in the cytoplasm; and within the dark-staining nucleoplasm of each cell, a jet-black nucleolus is present (Fig. 7). In sections of 100μ or more in thickness, a dark stain in the peripheral zone of the endosperm indicates a diffusion of the enzyme from the aleurone layer.

Preformed phosphate is apparent in minimal amount in the cytoplasm in control sections of the aleurone layer (Fig. 5).

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THE ACETOCARMINE SMEAR TECHNIC[1]

LUTHER SMITH, *State College of Washington, Pullman, Wash.*

ABSTRACT.—**The acetocarmine smear technic is described primarily for beginners. An effort was made to bring together into one paper the work-a-day details to be followed in collecting specimens and making preparations of plant chromosomes at prophase, of condensed chromosomes at meiosis and mitosis (including mitosis in microspores), and of coiled chromonemata. A schedule for converting temporary slides into permanent mounts is given, and a technic for making smears of the chromosomes in the salivary glands of Drosophila is described.**

INTRODUCTION

It is the hope and intent of the writer to prepare a paper with sufficient information to enable a novice to develop proficiency in preparing acetocarmine smears by following the instructions. However, the beginner should not be too easily pleased or displeased by the results of early efforts. The technic is something of an art, and as such requires patience and continued effort to equal or surpass the results of the masters of the art. It is also hoped that the paper may be helpful to those already using the technic, either in their own work or as a reference to which they can direct students.

Current acetocarmine smear procedures are based on the method as developed by Belling (1926) and improved by McClintock (1929 and unpublished), and others. The technic has been used successfully on a wide variety of plant and animal material, such as anthers, root tips, embryonic leaves, buds, salivary glands of Diptera (Painter, 1934), various embryonic tissues of insects (Carlson, 1942; Smith, 1943), and testes and embryonic tissues of birds and mammals (Painter, 1939). Descriptions of the technic as it is applied to certain species are given herein. Adaptations to additional species may be found in the references (which were selected more for details on

[1]Published as Scientific Paper No. 687, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

The author is indebted to Dr. A. B. Griffin of the Department of Zoology, University of Missouri for the section on smears of salivary gland chromosomes in *Drosophila*. He also desires to acknowledge indebtedness to Drs. C. R. Burnham and Barbara McClintock from whom he learned the technic, and to these and Drs. H. C. Aase, C. L. Huskins, W. E. Maneval, Karl Sax, and others for suggestions incorporated in the manuscript.

technic than for precedence) and in other papers in the literature, many of which were cited by Darlington and La Cour (1942).

General Information and Instructions

There is considerable variability among carmine dyes. The National Aniline and Coleman and Bell carmines have been tested and recommended.

Acetocarmine is prepared by boiling an excess of carmine in 45% acetic acid (about ½ gram in 100 ml.) for 2 to 10 minutes or in a reflux condenser or stoppered bottle for ½ hour or more. After cooling, the acetocarmine is filtered and used full strength for staining pachytene preparations but may be diluted with 45% acetic acid for condensed chromosomes. Thomas (1940) recommended using 1 part stain to 2 parts of 45% acetic acid for chromosomes of fruit trees. Sometimes it is necessary to dilute or refilter old batches of stain. A good batch seems to be a matter of luck, and one just continues to make stain until a product that gives satisfactory staining is obtained. A good stain, properly used, results in darkly stained chromosomes with almost clear centromeres, and nearly colorless cytoplasm. Once obtained, a good stain remains good for years.

Care must be exercised in the amount of stain used in preparing smears. Pachytene preparations of maize (and related plants) are usually better if the drop of carmine is of such size that it barely reaches the edges of the cover slip. The stain draws the cover slip down closely to the slide, flattening the large microsporocytes without breaking the chromosomes. On the other hand, slides of condensed chromosomes are usually better if prepared with a larger volume of stain and the excess pressed out on a blotter, simultaneously flattening the cells and spreading the chromosomes. In examining newly made slides of root tips it is particularly important to have enough stain under the cover slip so that effective flattening of individual cells with pressure from a needle can be accomplished.

Additional flattening of individual cells (especially important for photomicrography) may be obtained by applying pressure to the cover slip over the cell for a day or longer. This pressure can be applied by placing a small piece of paper over the cell and weighting it down with weights, or by applying pressure with a spring-type clothespin.

If slides are to be examined later, they may be sealed around the edges of the cover slip with a sediment-free mixture (equal parts) of gum-mastic and hard paraffin. Paraffin alone will do but not so well. A satisfactory device for applying the seal can be made from a wire test tube holder or from a piece of copper wire. Another, water-

soluble seal of the following (or similar) composition has been used by various workers:

Distilled water	90 ml.
Glycerin	16 ml.
Gum arabic	50 g.
Chloral hydrate	100 g.

H. C. Aase and M. Owenby have used this seal in the dry climate of Pullman, Washington, and found that many slides remain usable for a year or longer. The stain is allowed to evaporate (if necessary) until it barely reaches the edges of the cover slip; then the seal is applied along the edges of the cover with a medicine dropper. A weight (*e. g.* a bolt nut) is placed on the cover slip to prevent the seal from flowing under it.

For many purposes, slides can be examined, even using the oil immersion objective, immediately or within a few hours after preparation and discarded without sealing.

Good temporary smears usually improve for a day or longer (although poor slides get worse) and may remain good for a month or more. Therefore, it is often desirable to wait a day or so before examining a slide thoroughly, before making the mount permanent, or before taking photomicrographs.

Storing slides in a refrigerator is not satisfactory because frequently contraction breaks the seal, allowing the smears to dry up.

The spindle is often not clear in acetocarmine preparations, but may be fairly apparent in material fixed for a long time or in slides that are old.

Nucleoli are reasonably clear in acetocarmine smears.

Aceto-orcein (1 gram orcein dissolved in 45 ml. of hot glacial acetic acid, and diluted when cool with 55 ml. of distilled water) can be used in the same way as acetocarmine (La Cour, 1941) and for some materials has certain advantages. It is not so likely as acetocarmine to overstain the chromosomes and does not stain the cytoplasm or nucleoli.

Perhaps it should be mentioned that especially satisfactory results are obtained in viewing and photographing acetocarmine smears if green or yellowish-green light filters (such as Eastman Wratten "M" Filters No. 58 and 56, alone or in combination, or No. 62 for maximum contrast) are used.

Fixatives and Fixation

Some materials may be satisfactorily fixed and stained with acetocarmine alone, but for most purposes better preparations are obtained if the material has been previously fixed. Fixation may vary

from a few minutes to several days depending upon the material, fixative, temperature, and purpose for which the slide is to be made. Fixation of condensed chromosomes is not so critical as fixation of prophase chromosomes and may be continued longer without serious loss of quality. The fixatives usually used are:

Farmer's fluid (freshly mixed)

95% Ethyl alcohol (or absolute)	3 parts
Glacial acetic acid	1 part

Carnoy's fluids (freshly mixed)

	A	B
95 Ethyl alcohol (or absolute)	6 parts	6 parts
Chloroform	3 parts	1 part
Glacial acetic acid	1 part	3 parts

Farmer's fluid is used for pachytene preparations of maize, sorghum, etc. Carnoy's fluid A (some workers prefer B) gives better preparations of the condensed chromosomes of wheat and many other species. It does not harden the cells so much; leaves the cytoplasm clearer; does not swell the chromosomes so much; and keeps the specimens usable longer than Farmer's.

There is considerable evidence that better fixation, resulting in less clumping of chromosomes, may be obtained if the specimens are small. Thus, in some difficult materials, better results may justify fixing individual anthers or making hand sections of the larger tissues.

Emsweller and Stuart (1944) described a method for obtaining better preparations of meiosis in plants with large or numerous chromosomes by the use of enzymes after fixation. They washed out the fixative and treated the material for 10 minutes to several hours in a 1% solution of Clarase (or other enzyme extracts) before staining, and obtained very promising results.

In taking specimens it should be remembered that in some species there are definite daily cycles of divisions. These cycles are not clear-cut in the cereals and many other plants, but material (microspores particularly) fixed a little before mid-day, or possibly earlier for meiosis, seems to have more nuclei undergoing division than material fixed at other times.

If the specimens are not to be used when properly fixed, they may sometimes be kept usable (up to a year or longer) if stored at below freezing temperatures in a refrigerator, and allowed to warm up for several hours before smears are made. The specimens can be transferred to 70% alcohol, but storing immediately after killing in the original fixative seems to be about as satisfactory. In neither case are the slides ordinarily as good as those obtained from freshly fixed specimens.

Procedures for Special Purposes

MEIOSIS

A. *Maize* (*teosinte, sorghums, etc.*). Specimens are taken about 2 weeks before pollen shedding would occur. The tassel, as felt through the layers of sheaths and leaves, should not feel hard (too young) or soft (too old) but like an inflated football—resilient. A 2- to 4-inch, vertical slit, barely to the tassel, is made with a razor blade, and 2 or more intact branches are removed with forceps. If it is desired that the tassel grow on to maturity, the wound should be carefully closed and covered with a band of gummed paper tape.

The tassel branches are fixed in 5 ml. or more (barely covered) of Farmer's fluid for 1 or 2 days. If fixed in 2:1 or 1:1 instead of 3:1, slides may be prepared sooner. The quality of the smear is strongly dependent on the proper degree of fixation. For smears of pachytene, the material is easily fixed too long for best preparations, and if not to be worked within 2 or 3 days should be stored in a refrigerator.

After fixation, smear preparations may be made as follows:

1. Place a branch of the tassel in a small quantity of the fixative in a petri dish (over black to improve visibility).
2. Dissect out one of the anthers in a spikelet and place it on a clean, dust-free slide. Fine-pointed iron or stainless steel dissecting needles (thoroughly cleaned in a drop of acetocarmine at the beginning of each slide-making session to remove rust and dried stain), with 1/8-inch of the end curved at about a right angle may be used.
3. Add a small drop of acetocarmine to the anther at once.
4. Cut the anther in two and gently press it (beginning at the uncut ends) with the curved point of a needle, forcing the pollen mother cells out.
5. Briefly examine the sporocytes for the proper stage under the low power of the microscope. For the beginner, it is desirable to put on a cover slip for more thorough study, but he should soon strive to recognize the stages in the uncovered drop.
6. If the desired stage is present, tap out more of the pollen mother cells, carefully remove the debris, and quickly put on a clean cover slip (previously readied). A 7/8″-square, No. 1 cover slip is usually recommended. If too much time is taken to determine the proper stage, crystals will form at the edges of the stain and the preparation is spoiled, unless the crystals can be dissolved with more stain. If the proper stage is not present, the drop of acetocarmine containing the anther can be wiped off with cheese-

cloth and an older or younger anther selected for smearing on the same slide.

7. Heat the preparation (over an alcohol lamp, gas burner, or electric light bulb) to near the boiling point a few times. Be careful not to expand the air bubbles unduly, and examine the preparation under the microscope after each heating for proper staining. If a speck of dust, etc. holds the cover slip up at one spot, local pressure with a dissecting needle (using care not to move the cover slip) may remedy the trouble.
8. After the mount has cooled, carefully seal.

In learning the technic, it is necessary to strive for speed. Later, when proficient, it is possible to work so fast that the staining may not be dark enough.

B. *Wheat (and related plants).* The whole spike is fixed just before the tips of the awns (in awned types) appear out of the boot of the last leaf; or when the sheath of the last leaf has emerged about half way. Fixation is in Carnoy's fluid for 2 to 5 days. The slides are prepared in the same way as recommended for maize, except usually all 3 anthers from a floret are used to make one slide, and a larger drop of stain is used.

C. *General.* After the technic has been mastered for a particular species, the problem of finding the proper stage for taking specimens and where the most advanced and latest anthers are to be found in the specimen usually presents no serious difficulty. However, in plants such as oats, where a regular series of stages is not evident, finding whether the specimen has anthers at the proper stage is laborious at best.

ROOT-TIP SMEARS

Farmer's or Carnoy's fluid, acting for 2 to 10 days, or even longer, gives good fixation.

1. Place about 1 mm. of the root tip on a slide and macerate in a small drop of acetocarmine with a spear-point needle or glass rod.
2. Add a drop of stain, cover (without prior examination under the microscope) and heat.
3. Flatten individual cells by applying pressure with the point of a dissecting needle while the cell is observed under the low power of the microscope.
4. Reheat, cool, and seal.

Some workers prefer to make thin hand-sections of the root tips with a razor blade before smearing. That may be helpful with larger root tips such as those of maize.

It is very important to get root tips in which cell division is going on rapidly. Thus, it is desirable, where possible, to take root tips from seeds just starting to germinate on blotters, because they are growing rapidly and are free of dirt. If tips are taken from plants growing in the soil, they must be carefully freed of grit (*e. g.*, with the fingers or lips) before fixing or smearing. Judicious flattening of selected cells in newly prepared slides under observation in the microscope is also important.

Pretreatment with paradichlorobenzene (Meyer, 1945) greatly improves the smear technic for chromosome counts and for studies of chromosome morphology (Fig. 1). The method as described above would be modified only to the extent of soaking the root tips (or

FIG. 1.* Somatic chromosomes from root tips pretreated for 2 hours at room temperature in a saturated aqueous solution of paradichlorobenzene (Meyer, 1945), fixed in Carnoy's fluid and stained with acetocarmine. Counting of the chromosomes is facilitated, and morphological characteristics are accentuated. A. Maize B. Barley (×850).

*The writer is indebted to Dr. J. G. O'Mara for the use of Fig. A.

other embryonic tissue) in a saturated, aqueous solution of paradichlorobenzene for 1 to 4 hours before fixing. According to Meyer the solution is "prepared by mixing about 5 or 10 g. paradichlorobenzene crystals and about 500 ml. distilled water in a stoppered bottle and holding at about 60°C. overnight. It is used at room temperature." It should also be possible to adapt the method for microspore divisions where the material is more favorable for study of chromosome morphology.

With some species better smears of root tips are obtained if the middle lamella holding the cells together is digested so that the cells separate freely. This has been accomplished by pretreatment of the fixed tissue with 10% HCl for 10 to 30 minutes (and returning to the fixative) prior to staining, and in various other ways (Warmke,

1935). Faberge (1945) recommended the use of digestive juices from the stomachs of snails for the same purpose.

MICROSPORE DIVISIONS

In maize, wheat, barley, and rye, specimens may be fixed for the first mitotic division in the pollen grain about 5 to 7 days after meiosis—at the stage the tassel (in maize) is emerging and starch is beginning to appear in the spores. The second division occurs 5 to 7 days later, after a considerable deposit of starch is present. Some of the starch may be "bleached out" by adding crystals of chloral hydrate to the acetocarmine (3–10 grams to 1 ml.). Some chloral hydrate is almost necessary for clear observations on the second division. However, if too much chloral hydrate is added, the chromosomes are fragmented and destained. Frequently, particularly for the second division, it is difficult to determine whether the proper

FIG. 2. Root-tip cell of barley. No pretreatment.

stage is present without putting on a cover slip and heating the slide.

Sax's method of staining microspore divisions in *Tradescantia* is to smear the material on a dry slide and fix in Farmer's fluid for 10 minutes. The fixative is then drained off and replaced with several drops of acetocarmine. A cover slip is put on, the mount heated, set aside for 10–15 minutes, reheated, pressed firmly under a sheet of bibulous drying paper (Cenco), cooled, and sealed.

In species in which nuclear divisions occur in the pollen tubes, acenapthene and colchicine were successfully used by Swanson (1940, 1942) for facilitating the study of chromosomes.

STAINING TO SHOW COILS

Chromonemata coils in contracted chromosomes may be stained effectively in unfixed specimens.

1. Place a flower of the material under study, *e. g.* a spike of rye, on a moist blotter in a petri dish; it will stay fresh for several hours.
2. Take part of an anther and find the proper stage in the usual manner by examining a few pollen mother cells in a drop of acetocarmine.
3. If the proper stage is present, press and carefully draw the remainder of the anther (or another anther from the same floret) along a clean slide leaving ribbons of sporocytes.

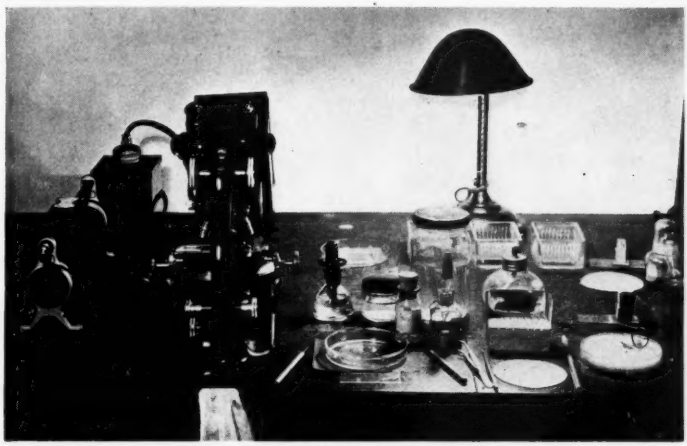

FIG. 3. Desk equipped for the aceto-carmine smear technic.
Left: microscopic equipment.
Right, front: Smearing apparatus.
Right, back: Apparatus for making permanent preparations.
For close-up of the right side of desk, see Fig. 4.

4. Pretreat the smear for 30 seconds or less in a solution of ammonia water in 30% alcohol (3 to 8 drops of concentrated ammonium hydroxide to 50 ml. of 30% alcohol).
5. Add a drop of acetocarmine.
6. Cover.
7. Heat, cool, and seal.

The pretreatment may be done in a staining jar or by putting a few drops of the solution on the slide as it lies on a table, removing the excess with blotting paper, held at the edge of the drop, or pouring it off. The manipulations must be done very carefully, so as not to damage the chromosomes.

If step 4 is omitted, ordinary acetocarmine smears from unfixed material are obtained.

The above method of staining to show coils was developed by Sax and Humphrey (1934), and was based on the discovery by Kuwada and Nakamura (1934) that ammonia accentuates the coiled structure of condensed chromosomes. Matsuura (1938) reported obtaining satisfactory slides for the study of coils merely by pretreating the

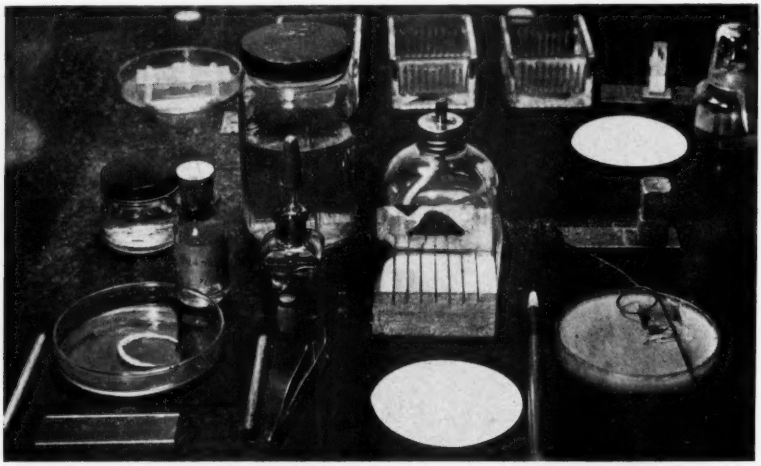

FIG. 4. Equipment used in making acetocarmine smears of plant tissues (front) and converting temporary to permanent slides (back row). In the front row are: 2 dissecting needles and a spear-point needle, slide, petri dish and specimen over black paper, forceps, blotter, glass-marking pencil, seal and applicator. For dry climates, at least, the water-soluble seal mentioned in the text is preferable. Second row: specimen bottle, acetocarmine, cover slip holder. Third row: cover slips and slides in jars containing alcohol, alcohol lamp, a sealed slide with lead weights to flatten an individual cell. Back row: a petri dish with a U-shaped glass rod supporting a slide, razor blade, 3 staining dishes, a slide with a clothespin pressing out excess balsam, balsam.

smears for ½–2 minutes in tap or distilled water. Boiling water, desiccation, and cyanides have been recommended as helpful in the study of coils also.

MAKING ACETOCARMINE SMEARS PERMANENT

If it is desired to convert temporary slides into permanent ones, the method of McClintock (1929) or Buck (1935) may be used, producing slides that will remain good for years, and probably in-

definitely. The following method of Sears (1941) gives excellent results with smears of maize, wheat, and barley.

1. Gently chip off the temporary seal with a razor blade. More of the seal can be removed with a small camel's hair brush wet with xylene (or 45% acetic acid for the water-soluble seal).
2. Soak the mount in equal parts of glacial acetic acid and 95% ethyl alcohol, until the cover slip comes off. (Process may be hastened by adding a little tertiary butyl alcohol to the solution.) Placing the slide upside down on a U-shaped glass rod in a petri dish (or in a porcelain dish with two ridges on the bottom) containing the solution is convenient.
3. Treat with equal parts of 95% ethyl alcohol and tertiary butyl alcohol for 2 minutes or longer, transferring the slide and cover slip to the staining dish containing the new solution and keeping them in the same relative positions. A split wooden match has been recommended as a convenient handle for transferring the cover slip.
4. Tertiary butyl alcohol, 1 or 2 changes, 2 minutes or longer in each.
5. Drain slide and cover slip (edges on a blotter) momentarily.
6. Apply a small drop of fairly thin balsam to slide and quickly reassemble slide and cover slip in same position as before. Press out excess balsam on a blotter.

Another method that is commonly used with smears of both plant and animal tissues is to follow steps 1 and 2 above, then run the slide and cover slip through 2 changes of 95% or absolute alcohol and mount in Euparal or Diaphane.

It is sometimes advisable to apply pressure to the cover slip for several days to force out the excess balsam. This can be done by weights or preferably by means of a spring-type clothespin that will hold the cover slip in place. A piece of glass, sheet metal, or stiff strap iron (slightly smaller than the cover slip) under the clothespin will equalize the pressure.

A considerable number of slides (50 or more) can be made permanent using the same solutions in Coplin jars, or preferably in staining jars in which the slides rest on edge, without replacing or replenishing. However, the alcohol-acetic-acid solutions should be mixed just before they are used.

More material will be retained on the slide and cover slip, and because of the residues of stain, it is easier to remount the cover slip in the original position, if the preparation is several days old before

it is made permanent. If the cover slip is inadvertently broken, the pieces can be remounted with reasonable satisfaction.

If the material, such as microspores, is likely to be lost in the process of making the slide permanent, the cover slip or slide may be coated with a thin layer of Mayer's egg albumen (possibly Haupt's Adhesive would be better) before the smear is made. Another possibility is to lay the slide and cover slip flat on a table, putting the solutions on with a medicine dropper and taking them off with blotting paper, held at the edge of the drop, or pouring them off.

If the preparation has become too darkly stained before being made permanent, it may be destained by running 45% acetic acid under the cover slip and heating.

A number of methods have been devised to prepare smears that are more or less permanent, thus eliminating the necessity of converting temporary mounts. Two of these involve the use of dioxane and white corn syrup (Hillary, 1938, 1939). Another involves the use of gelatin or other media (Zirkle, 1940). These methods are satisfactory for some materials but are not as widely applicable as the temporary smear technic.

SALIVARY GLAND PREPARATIONS OF DIPTERA

The preparation of salivary gland smears from Dipteran larvae (*Drosophila, Sciara, Chironomus, Simulian,* and others) is a common application of the acetocarmine smear technic. The procedure described here is recommended particularly for *Drosophila;* modifications required by other genera will suggest themselves to the investigator as he works.

An agar culture medium of any standard high-yield type (high brewer's yeast content) is used as the substrate for 2 to 6 pairs of adult flies. The adults are transferred to fresh culture vials at 6-hour intervals in order that an array of larvae of different ages may be available. Cultures are maintained at the standard temperature of 70–72°F. Larvae are ready for dissection when they emerge from the food mass and begin to crawl upon its surface or upon the sides of the container in preparation for pupation. As with pachytene preparations of maize, there is considerable variation among stocks as to the quality of smear preparations obtained.

1. With a small wire loop, transfer active larvae to a black dissecting dish containing 0.07% NaCl.
2. *Dissection.* Using two pairs of fine-pointed forceps, grasp the most anterior tip (oral hook region) of the larva with one hand and the posterior end of the body with the other. A

slight pull immediately separates the head region from the rest of the body, trailing out the intact salivary glands and the anterior portion of the alimentary canal. Maintaining a firm grip on the oral hook fragment, isolate the glands by breaking their common duct with the second pair of forceps, keeping the pair of glands united. Practice will result in accuracy and speed in dissection; note that these qualities cannot be achieved through the use of dissecting needles in place of forceps.

3. *Staining and fixing.* After several pairs of glands have been dissected, they may be transferred to a small dish of acetocarmine with a medicine dropper. The time required for staining varies markedly with different species and may be estimated only after experimentation. *D. melanogaster* ordinarily requires a staining period of 20 to 30 minutes.

4. *Smearing.* With a pipette, transfer a drop of acetocarmine and several pairs of glands to a clean slide; drop a cover glass on without regard for air bubbles, which will be eliminated during the smearing process, and blot the covered slide gently with filter paper. Under a dissecting microscope, press out the glands with a blunt needle, using sharp, light taps to rupture isolated cells. The desired degree of spreading is readily observed at a magnification of 30× or greater, since the salivary chromosomes are easily visible.

5. *Temporary mounts.* Seal, if the mount is to be used as a temporary slide.

6. *Permanent mounts.* Place fresh slides (or slides whose seal has been chipped off) in the vapor of 95% ethyl alcohol and allow 6 to 8 hours of vapor-dehydration as recommended by Bridges (1937). A small desiccator with alcohol below a slide-bearing shelf, or a Stender dish (lid sealed with vaseline) containing 5 ml. of alcohol may be used as the dehydrating vessel. After the dehydration period, the slides are immersed in 95% alcohol and may be allowed to remain for 1 to 12 hours. At the end of the immersion period, the slides are placed flat upon a paper towel without removal of excess alcohol. A drop of Euparal or Diaphane is placed at one side of the cover glass and is caused to flow gently under the cover by careful raising of the cover with a sharp needle; the opposite side of the cover should be held in position with the fingers during the process, since sliding of the cover tends to roll up and spoil the thin smear. To complete the mount, a drop of Euparal is placed at opposite edges

of the cover slip. The Euparal will be drawn under the cover slip as the alcohol evaporates, preventing air bubbles.

WASHING SMEAR SLIDES

Acetocarmine smear slides may be cleaned efficiently as follows:

1. Remove the paraffin-gum-mastic seal or immersion oil by soaking the mounts in xylene for about a week. The water-soluble seal recommended can be removed by soaking in water.
2. Separate the cover slips from the slides (a razor blade will be helpful in some cases.)
3. Wash the cover slips and slides separately in warm, (not hot) soapy water. Use care not to scratch the slides against one another.
4. Rinse in running water.
5. Store in 70% alcohol until used.

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LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOK AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

BOOK REVIEW

COOPER, HERBERT J. (Editor). **Scientific Instruments.** 5½ by 8½ inches. 305 pages. Illus. Chemical Publ. Co., Brooklyn. $6.00. 1946.

This book discusses a wide range of instruments, used in the laboratory, in the field, and in industry, for making physical measurements, not only describing the instruments themselves but explaining the fundamental principles on which they are based. The book is profusely illustrated with diagrams and photographs, and the language is clear and sufficiently non-technical so that one does not need to be a specialist in the field covered by any particular chapter in order to understand it. It is, perhaps, hardly a book for the layman; but it is certainly well designed to give any scientist a fair comprehension of the instruments used in fields of work other than his own. Fifteen collaborators are named as having contributed to the work; in fact it is obvious that no one scientist, without such collaboration, could have written intelligently about the instruments used in such a variety of different fields. Nevertheless, the style is so consistent throughout, as to suggest one hand doing most of the actual writing; presumably it is largely modesty that has inspired the use of the term "editor" on the title page.

The book is divided into five sections: Optical Instruments; Measuring Instruments; Navigational and Surveying Instruments; Liquid Testing; Miscellaneous (including calculating machines, vacuum tubes, etc.) The editor and all his collaborators are British; and it is often of interest to the American reader to note differences between the British approach to such subjects and that with which he is more familiar in the United States. Possibly the omission of one or two types of instruments (e.g. spectrophotometers) may be explained by the fact that different instruments are in use in the two countries.

Of special interest to readers of STAIN TECHNOLOGY is, naturally, the section on Optical Instruments, particularly the chapter on The Microscope and Electron Microscope. These subjects are adequately, although of necessity briefly, treated; the few pages on the electron microscope, for instance, can be recommended to anyone, even though unfamiliar with electronics, who wishes to obtain an understanding of this new and rather complicated instrument.—*H. J. Conn.*

MICROSCOPE AND OTHER APPARATUS

KINGMA BOLTJES, T. Y. (U. Amsterdam, Holland.) **Some experiments with blown glasses.** *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **7**, 61–76. 1941.

Experiments were carried out with blown glass spheres in order to ascertain whether the observations reported by van Leeuwenhoek might be made by means of these. Microphotographs clearly show that this is actually the case. Van Leeuwenhoek, however, mentions his use of ground lenses. At low magnification these are certainly better than blown glasses. The possibility of van Leeuwenhoek having used cover slips is discussed.—*M. P. Löhnis.* (Courtesy *Biological Abstracts*).

SHRYOCK, A., and ASHLEY, L. M. **Staining racks of three designs for coverglass preparations.** *J. Tech. Methods*, **25**, 63–6. 1945.

Staining racks in which up to sixty cover-glass smears can be safely stained at once are described. The racks are made of stainless steel which is resistant to most staining reagents. They measure approximately 68 mm. × 27 mm. × 24 mm. Smaller racks suitable for student use are also described.

The use of these racks conserves time, space, and reagents.—*C. Randall.*

VICKLUND, R. E. **Preventing the fungus fouling of optical instruments.** *Ind. Eng. Chem.*, **38**, 774–9. 1946.

Metallic foil containing 15 γ of radium per sq. in. in equilibrium with its decay products prevents fungus fouling of optical instruments. Lenses up to 3 in. in diameter can be adequately treated by surrounding each lens surface with a strip of foil, the width of which is equal to one-ninth of the lens radius. The use of radium-activated foil is economically feasible and presents no health hazards.—*R. T. Whittenberger.*

MICROTECHNIC IN GENERAL

BOURDON, H. **Nouveau procédé d'inclusion par les résines polyvyniliques.** *Bull. d'Histol. Appl.*, **20**, 46–8. 1943.

The author describes a new method of embedding with impregnation by vinyl acetate which solidifies by polymerization to a hard, colorless mass. Spontaneous polymerization may be avoided by adding 5% hydroquinone to the stock solution. The hydroquinone must be removed by distillation before using.

The recommended procedure is as follows: Fix in formalin, sublimate or a bichromate mixture. (Picric acid interferes with the polymerization reaction.) Dehydrate in four changes of dioxane over a period of 24 hr. Impregnate for 24 hr. with two changes of pure vinyl acetate. To prepare the embedding fluid, add 3% benzoyl peroxide to the vinyl acetate and boil to a sirupy consistency. Pour into a cellophane mold and add the impregnated tissue. Place in an oven at 37° C. for 48 hr., then at 56° C. for 48 hr. After cooling, immerse in water and remove the mold. The block may be mounted on a wooden base with the aid of a little melted cellophane, and sections may be cut as thin as 2 or 3 μ. Before staining, the embedding material may be dissolved in an organic solvent such as toluene.

The advantages of this method are that very thin sections may be obtained without tearing or distorting the tissue and that the sections adhere remarkably well to the slide. The disadvantages are the long and complicated procedure and the inability to obtain ribbons.—*Jean E. Conn.*

CARES, R. **A note on stored formaldehyde and its easy reconditioning.** *J. Tech. Methods*, **25**, 67–70. 1945.

Methods for overcoming the tendency of formaldehyde stock solutions to form paraformaldehyde by polymerization and formic acid by oxidation are given. To recondition formaldehyde which has become weakened due to polymerization the author recommends the following procedure: shake milky solution to suspend sediment; pour into Mason jars and seal tightly; autoclave at 15 lb. for 30 min.; cool. A water-clear solution results which may be stored or diluted for use.

Dilute (10%) solutions tend to form acid more readily than stock solutions (approx. 40%). Marble chips are useful for neutralizing diluted solutions.—*C. Randall.*

DELAUNAY, A. **La déshydration méthodique dans la technique histologique.** *Bull. d'Histol. Appl.*, **17**, 246–8. 1940.

The conventional methods of dehydration call for the tissue to be placed in the bottom of a vessel containing alcohol. Since water is heavier than alcohol, that leaving the tissue tends to remain at the bottom of the container. Thus the material to be dehydrated is always in the worst possible position. To avoid this, the author proposes a vessel, 35 or 40 mm. in diameter, with a widened base and a constriction in the middle which supports a non-corrosive wire gauze. The tissue is placed on the gauze with just enough alcohol in the vessel to cover its upper surface. This flask can be used in the same way for decalcification.—*Jean E. Conn.*

MURDOCK, S. E. **A method for the removal of precipitate from tissues fixed in formaldehyde.** *J. Tech. Methods*, **25**, 71–2. 1945.

A fine brownish-black crystalline pigment derived from laked blood frequently results from formaldehyde fixation. This may be overcome by the following

procedure: bring tissues to water; place in ammoniacal H_2O_2 solution (25 ml. 3% H_2O_2; 25 ml. acetone; 1 drop concentrated ammonia); let stand 1–2 hr. or until precipitate is removed; wash in water; stain as usual.—*C. Randall.*

DYES AND THEIR BIOLOGICAL USES

DAVID, W. A. L. **The quantity and distribution of spray collected by insects flying through insecticidal mists.** *Ann. Appl. Biol.*, **33**, 133–41. 1946.

In studying insecticides, it is helpful to measure the quantity of spray which flying insects accumulate from a mist dispersion. When the housefly (*Musca domestica* L.) and the yellow fever mosquito (*Aëdes aegypti* L.) are placed in a chamber containing a mist of odorless kerosene dyed with Sudan III, droplets accumulate on both surfaces of the wings but not on any other part of the body. The dye is later found in the gut, feces, and malpighian tubules. To determine quantitatively the amount of an insecticidal mist which is accumulated, the spray is dyed with a known quantity of the dye, the insects are later extracted in odorless kerosene, and the extracts are compared with a set of prepared standards. From the amount of dye extracted, the quantity of the insecticide accumulated is calculated by assuming that with relatively nonvolatile materials the ratio of dye to insecticide does not undergo any appreciable change. This colorimetric method is used to study the effect on the quantity of spray accumulated, of changes in the properties of the mist dispersions and in the behavior of the insects. Within limits the dye accumulated increases with the activity of the insect and the size of the mist particles.—*H. P. Riley.*

GOMORI, G. **Buffers in the range of pH 6.5 to 9.6.** *Proc. Soc. Exp. Biol. & Med.*, **62**, 33–4. 1946.

Three organic chemicals used separately with 0.1 **N** HCl were found to cover the range from pH 6.5 to 9.6. These substances are: (1) 2,4, 6-collidine (s-collidine) (Eastman Kodak Co.); (2) Tris (hydroxymethylaminomethane) and (3) 2-amino-2-methyl-1,3-propanediol (Commercial Solvents Corp.). They do not precipitate calcium salts and are inexpensive and stable. Values for pH and pK_b at 23° and 37° C. are given.—*H. A. Davenport.*

McBAIN, J. W., and GREEN, AGNES ANN. **Solubilization of water-insoluble dye in soap solutions: effects of added salts.** *J. Amer. Chem. Soc.*, **68**, 1731–6. 1946.

The solubilization of orange OT (F.D. and C. orange No. 2) in aqueous solutions of soaps at 25° C. is described. In sufficiently dilute solutions of detergents, there is no solubilization, presumably because there are no colloidal particles in which the solubilized material can be incorporated. As suitable micelles form in the detergent solution upon increase of concentration, the amount of dye solubilized by each mole of soap rises to a constant value, which increases again only in the highest concentrations. Solubilization is not proportionate to the amount of hydrocarbon in the soap molecules or micelles, but rises rapidly with a slight increase in hydrocarbon content. In all concentrations, salts (KCl, K_2SO_4, KSCN, which by themselves are non-solubilizing) greatly increase the amount of solubilization by a given amount of soap.—*R. T. Whittenberger.*

MICHAELIS, L., and GRANICK, S. **Metachromasy of basic dyestuffs.** *J. Amer. Chem. Soc.*, **67**, 1212–9. 1945.

Many basic dyestuffs are adsorbed by stainable substrates in two different shades of color, designated as the normal and the metachromatic color. As a model for a substrate stainable in the normal color, a 3% solution of nucleic acid at pH 4.6 is chosen; as a model for a substrate stainable in the metachromatic color, a solution of agar at pH 4.6 is chosen. All dyestuffs capable of metachromasy disobey Beer's law in aqueous solution, owing to the fact that with increasing concentration dimeric molecular aggregates of the dye molecules are formed. The absorption maximum of the dimer lies at shorter wave lengths than that of the monomer as it exists in dilute solution. In the presence of agar still higher molecular aggregates are formed and are absorbed by agar. It is probable that one cation of the dye is adsorbed primarily by an acid side chain

of the substrate and carries along an aggregate of other dyestuff molecules including their anions. The fact that metachromatic color in agar gives way, reversibly, to an approximately normal color at higher temperature corroborates the hypothesis of polymerization of the dye molecules. The absorption bands of these high polymers are displaced toward shorter wave lengths and are more diffuse than those of the monomer. The metachromatic effect is nearly independent of pH and cannot be attributed to formation of the free base. In contrast, in the presence of nucleic acid the absorption spectrum of all basic dyes is independent of the concentration of the dye and is similar to that of the dye in dilute solution. Beer's law is strictly obeyed. The peak of absorption is displaced slightly toward longer wave lengths by nucleic acid and the peak is lowered slightly. No polymerization takes place. Each cation of the dye is combined with one acidic side chain of nucleic acid to form a stoichiometrically well-defined salt-like compound. Little is known about the correlation of the chemical structure of a dye with its faculty of polymerization. Yet this faculty of polymerization is always correlated with the metachromatic effect.—*R. T. Whittenberger.*

OAKLEY, C. L., WARRACK, G. H., and VAN HEYNINGEN, W. E. **The collagenase (κ toxin of Cl. Welchii type A).** *J. Path. & Bact.*, **58**, 229–35. 1946.

The demonstration of collagenase is facilitated by diazotizing collagen to form a dye which, by going into solution, measures collagen digestion. The collagen-dye is prepared as follows: screen 1 lb. of hide powder (Baird & Tatlock) to 60 mesh; about 120 g. of screenings should be obtained. To a solution of 0.575 g. benzidine in 100 ml. of water containing 3 ml. concentrated HCl, cooled in an ice bath, add slowly 0.45 g. $NaNO_2$ in 10 ml. of water. Allow to stand 10 min., then pour the tetrazotized benzidine into a chilled solution of 6.25 g. of crystalline sodium acetate in 500 ml. of water. To the mixture add with constant stirring 1.1 g. R-salt (Na salt of 2-naphthol-3,6-disulfonic acid) in 100 ml. of water followed by 20 ml. *N* K_2CO_3. A brick-red dye is formed. Meanwhile wash 80 g. 60-mesh hide powder by repeated suspension in and filtration from 10 liters of water and finally resuspend in 500 ml. water containing 30 ml. 2*N* K_2CO_3. Add the dye to the suspension in 6 equal lots at 10 min. intervals. The color changes from brick-red to purple-red. After all dye has been added, add 25 ml. 2*N* K_2CO_3 and allow mixture to stand 10 min. Centrifuge or filter off the dyed hide powder and wash by resuspending 5 times in 5-liter quantities of water. Resuspend in 300 ml. of water, stirring constantly, and slowly add 1.7 liters of acetone. Repeat the acetone washings until the washings are no longer pink, filter off the dyed hide powder ("azocoll") and remove residual acetone at 37° C. Screen the azocoll to 60 mesh, dry in P_2O_5 under reduced pressure and keep in 5–10 g. amounts in rubber-capped bottles under nitrogen.

Azocoll required as indicator should be suspended as needed in 1% Manucol IV (a polymer of *d*-mannuronic acid, Allbright & Wilson, Birmingham).—*S. H. Hutner.*

ANIMAL MICROTECHNIC

CAMPBELL, BERRY, and NOVICK, ROSALIND. **A quantitative method for the study of chromatolysis.** *Proc. Soc. Exp. Biol & Med.*, **61**, 425–7. 1946.

Six cell types are defined accurately and examples are given of their use in quantitative evaluation of cellular changes in the spinal cords of cats after root section. The sequence of changes is illustrated by charts and figures. The method differentiates chromatolytic changes which have gone on for varying periods of time.—*H. A. Davenport.*

DILLER, I. C. **Smear methods for mammalian tissues, including tumors.** *J. Tech. Methods*, **25**, 73–6. 1945.

Acetic orcein is very satisfactory for nuclear preparations of soft mammalian tissues, especially rapidly growing tumors. For tumor tissue, the method is as follows: tease bits of tissue into minute fragments with fine needles in a drop of acetic orcein (1 g. orcein dissolved in 45 ml. boiling glacial acetic acid; 55 ml. distilled water; cool and filter); press cover glass firmly down upon the prepara-

tion and complete dissociation of cells by tapping with a blunt instrument; store overnight in vapor chamber (95% alcohol); soak off cover glass in fluid alcohol; counterstain in fast green; rinse in two changes of 95% alcohol; mount in diaphane. Details in the cell body are sharper if the tissue is teased in physiological saline solution until it is semifluid. These preparations may be floated on any fixative. The disadvantage of the saline method is that fresh tissues must be prepared at once.—*C. Randall.*

GLEES, P., MEYER, A., and MEYER, M. **Terminal degeneration in the frontal cortex of the rabbit following the interruption of afferent fibers.** *J. Anat.*, **80**, 101–7. 1946.

One of the authors (Glees) has developed an impregnation technic which he found very useful in the demonstration of the degenerating unmyelinated nerve fibers and terminals which were studied in this work. The technic consists in fixing a block of tissue for at least 5 days in 5% formol-saline, then cutting on the freezing microtome at 12–15 μ. The sections are washed well in distilled water and left for 24 hours in 50% alcohol with 5–10 drops of concentrated ammonia, re-washed in distilled water and placed in 10% $AgNO_3$, where they are left until brown (24 hours—5 days). Then, without washing, they are reduced in several changes of 4% formaldehyde (i.e. commercial formalin 1:10, made up with tap water). From this the sections are placed in the following ammoniacal silver solution: 10 ml. absolute alcohol; 10 ml. 20% $AgNO_3$; ammonia added until the precipitate so formed is redissolved. Sometimes a few additional drops of ammonia seem to be needed to obtain the result desired. The sections remain in this solution for ½–1 minute. The reduction in several changes of 4% formaldehyde is repeated; the sections are washed twice in distilled water, fixed in 5% hypo, washed well, dehydrated in graded alcohols, cleared in creosote, and mounted.—*Warren Andrew.*

HUSEBY, ROBERT A. **Hydroxybenzene compounds as cytoplasmic fixatives.** *Proc. Soc. Exp. Biol. & Med.*, **61**, 122–5. 1946.

Mitochondria, secretion granules and other specific types of granulation are well preserved in tissues fixed in neutralized formalin-pyrogallol and formalin-resorcinol mixtures. Pyrogallol is added to 10% formalin in 3.5 to 7% concentrations; resorcinol in 4.5 to 9% additions. Both mixtures are alkalinized with 1% by volume of 1 *N* NaOH. Fixation time of 6 to 24 hr. is recommended. A discussion of staining granules in cytoplasm by several technics is given and illustrated by two figures. Hydroquinone and catechol mixtures are less effective than pyrogallol or resorcinol. This type of fixation is of special interest in connection with the centrifugal fractionation of cellular components and in tissue culture.—*H. A. Davenport.*

KELLER, G. J. **A reliable Nissl method.** *J. Tech. Methods*, **25**, 77–8. 1945.

The following method overcomes the difficulty of demonstrating chromophilic substance of nerve cells in material in which fixation has been poorly controlled: bring sections to distilled water; 0.5% cresyl violet (Coleman and Bell or Grübler's), 3–5 min.; 2 changes of distilled water; 95% alcohol, 30 sec.; absolute alcohol, 30 sec.; xylene, 1 min.; equal parts of Canada balsam and xylene, 2 min.; absolute alcohol, 10–30 sec.; several changes of xylene. The last three steps may be repeated if differentiation is not complete. Carbol-xylene cannot be substituted for xylene.—*C. Randall.*

MacNEAL, W. J., and BLEVINS, A. **Fixation of tissues by perfusion technique.** *J. Tech. Methods*, **25**, 54–60. 1945.

Fixation of tissues by perfusion technic is recommended as a supplementary method of preparing tissues for microscopic study. The authors prefer Helly's fluid ($K_2Cr_2O_7$, 2.5 g.; Na_2SO_4, 1 g.; $HgCl_2$, 5 g.; water, 100 ml.; 40% formaldehyde, 5 ml.) which causes prompt fixation.

A surgical specimen may be quickly weighed and measured, a portion removed for culture and one for fixation while in undistended state. The remainder of the specimen may be promptly perfused to remove blood and to fix the structures while the vascular spaces are distended. Finer details of structure may sometimes be more distinctly seen in tissues fixed by arterial perfusion.—*C. Randall.*

PAUL, PAULINE, and McLEAN, BETH BAILEY. **Studies on veal. I. Effect of different internal temperatures on veal roasts from calves of three different weights.** *Food Research*, **11**, 107–15. 1946.

Histological Studies: Cooked muscles, 71° C. (160° F.), were sectioned on the freezing microtome at a thickness of 160 μ, and were stained with gentian violet to show the general distribution of connective tissue and to determine the arrangement of muscle fibers. Small pieces of raw muscle and of muscle cooked to 71 and 88° C. (160 and 190° F.) were fixed in formalin and Zenker's fixative. The Zenker-fixed pieces were embedded, sectioned, and stained with Mallory's triple connective tissue stain, while the formalin-fixed pieces were embedded, sectioned, and stained with Weigert's elastic connective tissue stain.

The changes in microscopic appearance brought about by cooking were (1) swelling of the collagenous fibers observed in the semitendinosus muscle, (2) partial breakdown of the connective tissue reticulum surrounding the muscle fibers, and (3) changes in the staining properties of the sections with the connective tissue of the cooked sample staining less intensively.—*William G. Walter.*

SALAZAR, A. L. **Résumé des travaux realisés depuis 1941 jusqu'a 1945.** *Centro de estudos microscópicos do Instituto para a Alta Cultura, Université de Porto.* Porto, Portugal. 23 pp. 1946.

Salazar has summarized in French the work which has been done on cell structures and especially on connective tissue by the iron tannate method. Almost all of the papers are from his laboratory and were written in Portugese so that such a summary is valuable to those who cannot read this language. A number of articles which have appeared in countries other than Portugal are included but the list is not complete. The bibliography will be found very useful to all users of the Salazar technic as well as to cytologists and animal histologists.—*B. F. Lutman.*

PLANT MICROTECHNIC

GUILLIERMOND, A. **La coloration vitale des chondriosomes.** *Bull. d'Histol. Appl.*, **17**, 225–37. 1940.

The author has studied in some detail the vital staining of the higher plants, Saprolegnia, and fungi, with particular reference to the vital staining of the chondriosomes, and has observed that dyes which stained chondriosomes in living cells when mounted on a slide would stain permanently nothing but the vacuoles in actively growing cells. The dye might stain the chondriosomes temporarily, but would soon be excreted into the vacuoles. The higher plants would grow normally with dye in the vacuoles, but the fungi would not grow until all the dye had been excreted from the plant. He concludes that the cells on the slide are in an abnormal condition and that staining of chondriosomes is not compatible with cellular growth.—*Jean E. Conn.*

MICROÖRGANISMS

HENKE, R. J. **Experiments on the differential staining method of Dold.** *Zentbl. Bakt., I Abt. Orig.*, **148**, 132–46. 1941.

In Dold's method bacteria are stained with phenol-aniline green solution, then treated with a Lugol solution and decolorized in an alcoholic urea solution, which is followed by staining with Bismarck brown. When treated in this way some of the Gram-positive bacteria take the counterstain, others keep the original green color. The writer proves in a series of experiments that it is not the urea compound which is responsible for the action of the decolorizing agent. It is a special proportion between water and alcohol which causes this effect. So when smears with Gram-positive bacteria stained by a special coloring agent are treated with a solution of 88 vol. of alcohol and 12 vol. of water for 5 min., the Gram-positive group of bacteria can be differentiated into Dold-positive and Dold-negative forms. A certain group of bacteria is in a borderline position and minor changes in the technic may cause an irregular result.—*A. C. Buys.* (Courtesy *Biological Abstracts*).

JOHNSON, EDWIN A. **An improved slide culture technic for the study and identification of pathogenic fungi.** *J. Bact.*, **51**, 689–94. 1946.

Sterile slide cultures of fungi on potato-dextrose agar formed good spores which adhered to the cover slip when it was removed carefully. These spore clusters were fixed by flaming, stained with 0.5% safranin (no specifications), and mounted in Clarite or balsam. Several species of fungi ordinarily difficult to identify were easily classified from such cultures.—*Virgene Kavanagh.*

KOPEL, M. **A modification of the Giemsa stain for malarial parasites.** *J. Tech. Methods*, **25**, 61–2. 1945.

Absolute ethyl alcohol can be substituted for absolute methyl alcohol in the Giemsa stain for malaria parasites, both in fixing the smear, and in the stain itself. Nuclei stain red and cytoplasm blue. The results of the modification are adequate and dependable.—*C. Randall.*

LAMANNA, CARL. **The nature of the acid-fast stain.** *J. Bact.*, **52**, 99–103. 1946.

Several experiments in staining bacteria with Ziehl-Neelsen's carbol fuchsin suggested to the author that the beading obtained resulted from the separation of the phenol and dye as a liquid phase. On this basis acid-fastness lies in the greater solubility of the phenol and dye in the cell constituents than in the decolorizing agent.—*Virgene Kavanagh.*

HISTOCHEMISTRY

McCRONE, W., SMEDAL, ANNETTE, and GILPIN, V. **Determination of 2,2-bis-p-chlorophenyl-l,l,l-trichloroethane in technical DDT. A microscopical method.** *Ind. Eng. Chem., Anal. Ed.*, **18**, 578–82. 1946.

The method depends on the influence of impurities on the rate of crystallization of the major component from the melt. It is applicable to a large number of important systems such as explosives, pharmaceuticals, dye intermediates, or almost any organic reaction product. As applied to the determination of p,p′-DDT in technical DDT, the method makes possible an analysis in 10 to 15 min. with an accuracy of ±0.5%. A small sample is heated on a hot plate to a temp. not exceeding 120° C. The melt is mixed by stirring for about 1 min. One drop is placed on a slide, and a cover glass is pressed down firmly to give a thin film. If necessary, the sample is seeded at the edge of the cover glass with a crystal of p,p′-DDT to initiate growth. The sample is allowed to cool for 2 min. and a thermometer is placed on the microscope stage with the bulb near the sample. After 2 more min. the temperature is recorded and the crystal front focused with the eyepiece micrometer scale. The rate of growth of the p.p′-DDT is determined over a period of 5 to 10 min. The temperature is noted several times during the determination. The rate of growth expressed in microns in 5 min. and the average temperature are used in a standard graph to determine the per cent of p,p′-DDT.—*R. T. Whittenberger.*